



## Original Scientific Article

**FREQUENCY OF CONTAMINATION WITH LISTERIA MONOCYTOGENES OF RAW DRIED CURED VACUUM PACKED SAUSAGES**Hristo Daskalov<sup>1</sup>, Fejzulla Fejzullah<sup>2</sup>, Alexandra Daskalova<sup>3</sup><sup>1</sup>*National Diagnostic and Research Veterinary Institute, BFSA, 1606 Sofia, Bulgaria*<sup>2</sup>*State University of Tetovo, 1200 Tetovo, Macedonia*<sup>3</sup>*Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria*

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**ABSTRACT**

The aim of this study was to collect actual data concerning the frequency of contamination with *Listeria monocytogenes* of some raw dried cured vacuum packed sausages, which are very popular in Bulgaria produced from Oct. 2004 till May 2008. 148 vacuum-packed samples were taken from 9 different food business operators during all seasons of the year. The samples were analyzed according to the USDA method for meat foods. Ten specimens were positive for presence of *Listeria monocytogenes*, which is equal to 6,75% of all tested samples. In two other raw dried cured sausages *L. welshimeri* and *L. innocua* were found, but these species are not pathogenic for consumers. In the period before the official implementation of the HACCP system (01.01.2006) in Bulgaria, 52 samples were examined and 5 *Listeria monocytogenes* isolates were found (~10%). 2,5 years after the HACCP implementation, 96 specimens from the same meat factories were tested and 5 *Listeria monocytogenes* isolates (5,2%) were detected. Samples taken from lots, produced in winter time were contaminated with *Listeria monocytogenes* more often (7 of all 10) than specimens taken during other seasons. Data was discussed through the point of view of the effectiveness of hygienic practices and HACCP system application. Also, application of the 'microbiological criterion' set in the COMMISSION REGULATION (EC) No 2073/2005 for ready-to-eat foods unable to support the growth of *L. monocytogenes* was considered.

**Key words:** *L. monocytogenes*; raw dried sausage; contamination; HACCP**INTRODUCTION**

Raw cured shelf-stable meat sausages are very popular in Bulgaria. This group of products includes some low-acid dried sausages and high-acid fermented sausages with high microbial stability (9). The significance of *Listeria monocytogenes* as a foodborne pathogen is complex. The severity and case-fatality rate of the disease require appropriate preventive measures, but the characteristics of the bacteria make it unrealistic to expect that all food

could be *Listeria*-free (16). According to Data (4), *L. monocytogenes* was isolated from various acidic foods, including sausages. The author noted that *L. monocytogenes* could be inactivated more easily by the stomach and intestinal pH and hence it would have higher infective dose, compared to some other food-borne pathogens. Recently it was noted that *L. monocytogenes* could possibly grow in sausage mix if there was fermentation failure (8). Also it was emphasized that during normal fermentation, drying and ripening there was a 10 to 100-fold decrease in *L. monocytogenes* counts. A low number of microorganisms could be found in the finished product which would not be able to multiply (11, 12). A possible benefit of the microbiological risk assessment (MRA) document for *Listeria monocytogenes*, developed jointly by the USDA and the USDA for risk ranking of ready-to-eat foods in 20 food categories (one of them is raw cured dried meat products) was noted (7). There is opinion mentioned that the primary sources responsible for

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introduction of *L. monocytogenes* into the processing environment had not been clearly identified (10). In the case of processing of raw cured dried meat products, contamination of sausage ingredients played the main role.

Raw cured dried meat products can be included into the group of ready-to-eat (RTE) foods unable to support the growth of *L. monocytogenes*, set in Chapter 1. "Food safety criteria" of the Commission Regulation (EC) No 2073/2005 in case of examination of pH and water activity ( $a_w$ ) of the tested batch. EFSA opinion (6) concluded that the actual data did not provide a clear picture of the risk of listeriosis. Based on the dose-response relationship of *L. monocytogenes*, the risk of listeriosis is mostly related to products with high concentrations of the pathogen. Thus it is important to analyze the actual concentration of the pathogen in RTE products and especially the percentage of products with high concentrations.

The aim of this study was to collect actual data concerning the frequency of contamination with *Listeria monocytogenes* of some raw dried cured vacuum packed sausages very popular in Bulgaria, produced from Oct. 2004 till May 2008 in different meat producing plants.

packed in meat factories and prepared for sale without any other treatments. All samples were taken from 9 different food business operators during all seasons included in the period of study (Oct. 2004 – May 2008). All tested specimens were kept at storage temperature from 0 to 4°C. The samples were transported and received at the laboratory up to 72 hours after processing.

### Microbiological analysis

The samples were analyzed according to the USDA method for meat foods, described by Ryser and Donnelly (15). Five colonies testing positive on PALCAM agar (Merck, Darmstadt) were taken and reinoculated on TSAYE agar (Merck, Darmstadt). Further examination comprised Gram staining, motility at 20-25°C, growth at 35°C, catalase activity (Hydrogen peroxide, Merck, Darmstadt), oxidase reaction (Oxidase reagent, bioMerieux) and  $\beta$ -hemolysis on blood agar (Merck, Darmstadt). Additionally, biochemical identification with API *Listeria* ID strip (bioMerieux, Inc., Hazelwood, Mo.) was done to all *L. monocytogenes* isolates.

## RESULTS

Data showing the detected *Listeria* spp. strains in raw cured dried meat products are presented in Table 1.

## MATERIALS AND METHODS

### Sampling

148 different kinds of raw cured dried meat sausages were studied. All of them were vacuum-

**Table 1.** Frequency of detection of *Listeria* spp. in raw cured dried meat products

Period of time	Season	Samples (n)	Negative specimens for <i>Listeria</i> spp.	Positive specimens for <i>Listeria</i> spp.	Species of <i>Listeria</i>
01.10. 2004 - 31.12. 2005 (before the official implementation of HACCP)	Autumn	12	11	1	<i>L. monocytogenes</i>
	Winter	13	11	2	<i>L. monocytogenes</i>
	Spring	9	9	0	
	Summer	10	9	1	<i>L. monocytogenes</i>
	Autumn	8	7	1	<i>L. monocytogenes</i>
01.01.2006 - 31.05.2008	Winter	12	9	3	<i>L. monocytogenes</i>
	Spring	13	12	0	
	Summer	11	11	0	
	Autumn	11	11	0	
	Winter	12	10	2	<i>L. monocytogenes</i>
	Spring	12	11	1	<i>L. innocua</i>
	Summer	12	11	1	<i>L. welshimeri</i>
	Autumn	13	13	0	
				12 (8,1 %)	<i>L. monocytogenes</i>
01.10.2004 – 31.05.2008		148 (100 %)	136 (91,9 %)	6,75% positive for <i>L. monocytogenes</i>	<i>L. innocua</i> <i>L. welshimeri</i>

Ten specimens were positive for presence of *Listeria monocytogenes*, equal to 6,75% of the 148 tested samples. In two other raw dried cured sausages *L.welshimeri* and *L.innocua* were found, but these species are not pathogenic for consumers. In the period before the official implementation of HACCP system (01.01.2006) in Bulgaria, 52 samples were examined and 5 *Listeria monocytogenes* isolates were found (~10%). 2,5 years after the HACCP implementation, 96 specimens from the same meat factories were tested and 5 *Listeria monocytogenes* isolates (5,2%) were detected. Samples taken from lots, produced in winter time were contaminated with *Listeria monocytogenes* more often (7 of all 10) than specimens taken during other seasons.

Results for morphological and biochemical characteristics of isolated *Listeria* spp. strains and their biochemical profile are shown in Table 2.

## DISCUSSION

Results of the study showed presence of *L.monocytogenes* and some other representatives of *Listeria* spp. in ready-to-eat raw cured dried sausages, produced in Bulgarian meat plants. Frequency of reporting of *L.monocytogenes* was 6,75% of all tested samples.

On the other hand specimens examined before the HACCP implementation showed higher frequency of contamination (9,6%), compared to samples tested after application of this system (5,2%). All 10 isolates had similar characteristics and biochemical profile. One of the tested samples was positive for *L.innocua* and another showed presence of *L.welshimeri*. In Brazil there was evaluation of raw cured meat products for occurrence of *Listeria monocytogenes*. The pathogen was detected

**Table 2.** Morphological and biochemical characteristics of *Listeria* spp. isolated from raw cured dried meat products

Species of <i>Listeria</i> (Number of isolated strains)	Gram staining	$\beta$ -hemolysis	Catalase activity	Motility at 20-25°C	Growth at 35°C	Oxidase reaction
<i>L.monocytogenes</i> (10)	+	+	+	+	+	—
<i>L.innocua</i> (1)	+	—	+	+	+	—
<i>L.welshimeri</i> (1)	+	—	+	+	+	—

**API *Listeria* biochemical profile of *Listeria* spp. isolated from the tested food samples**

	DIM	ESC	$\alpha$ MAN	DARL	XYL	RHA	MDG	RIB	G1P	TAG	$\beta$ HEM	Profile
<i>L.monocytogenes</i>	-	+	+	+	-	+	+	-	-	-	+	<b>6 510</b>
<i>L.innocua</i>	+	+	+	+	-	+	+	-	-	-	-	<b>7510</b>
<i>L.welshimeri</i>	+	+	+	+	+	+	+	-	-	+	-	<b>7711</b>

**\*Legend:** DIM – enzymatic substrate; ESC – esculin;  $\alpha$ MAN – 4-nitrophenyl- $\alpha$ D-manopyranoside; DARL – D-Arabitol; XYL – D-Xylose; RHA – L-Rhamnose; MDG – methyl- $\alpha$ D-glucopyranoside; RIB – D-Ribose; G1P – Glucose-1-phosphate; TAG – D-tagatose;  $\beta$ HEM –  $\beta$ -haemolytic activity

All *L.monocytogenes* isolates had similar morphological and biochemical characteristics and when tested with API *Listeria*, the biochemical profile was 6 510. *L.innocua* isolate showed lack of  $\beta$ -hemolytic ability, positive reaction to DIM, negative to XYL, RIB, G1P, and TAG, and biochemical profile 7 510. *L.welshimeri* isolate did not present  $\beta$ -hemolytic activity and manifested positive result to DIM, negative reaction to RIB and G1P and biochemical profile 7 711.

in 13.3% of Italian type samples of salami, while *L. innocua* occurred in 6.5% of the Italian type and in 16.6% of the Milanese type (2). In Turkey *Listeria* spp. and *L. monocytogenes* from 63 (21%) and 35 (11.6%) of the tested Turkish style fermented sausages (sucuk) (3). In USA it was proven that the cumulative 3-y *L.monocytogenes* prevalence for dry and semidry fermented sausages was 3.25%. This kind of sausages is very popular also in Bulgaria and 4 of 10 of our *L.monocytogenes* isolates were isolated from this type of raw

cured dried product (13). Opinion was noted that processing facilities were frequently contaminated with nonpersistent or persistent *L. monocytogenes*, which played an important role in contamination of the products. The contamination status of processing lines and machines was influenced by the compartmentalization of the processing line, with poor compartmentalization increasing the risk of *L. monocytogenes* presence (14). Data was presented that *L. monocytogenes* had the ability to adhere to stainless steel, but significant differences existed in the ability of various *L. monocytogenes* strains to attach to the surface.

Dust contaminated with *L. monocytogenes* was another source of contamination (5). Some results remarked that food safety incidents due to the growth of pathogenic bacteria such as *Listeria monocytogenes* in fermented sausage might provoke serious consequences for consumers (17).

To protect consumers from *L. monocytogenes* in ready-to-eat foods unable to support its growth, a 'microbiological criterion' set in the COMMISSION REGULATION (EC) No 2073/2005 for maximum level of 100 CFU/g products during their shelf-life was applied.

## CONCLUSION

Frequency of contamination with *L. monocytogenes* of the tested raw cured dried sausages for the study period was 6,75%. The level of contamination was higher in the period before the implementation of the HACCP system in meat producing plants. Winter season was the time with higher prevalence of *L. monocytogenes*. Rarely some other *Listeria* spp. were found.

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